Guidance to the Peer Review Panel for Evaluation of the Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Toxicity Test Methods

Questions to the Panel

A. *In Vitro* Acute Toxicity Test Methods Draft BRD

1) For each Section of the BRD:
   - Is the information provided adequate for its purpose? If not, what additional information is needed?
   - Are there any revisions that are needed? If so, then what are they?
   - Is there additional information or are there additional data analyses or comparisons that should be considered? If so, what are they?
   - Are there data gaps where other data are likely to be available but have not been provided? If so, what are the data gaps and how might these data be obtained?
2) In addition to using the NRU test methods as part of a weight-of-evidence approach for estimating starting doses, are there other uses of the NRU test methods that should be considered and evaluated?
3) In Section 3 of the BRD, do the substances selected to evaluate the reliability (intralaboratory repeatability and intra- and inter-laboratory reproducibility) and accuracy of the NRU test methods adequately represent the range of possible test outcomes, the range of possible chemical and physical properties of substances that might be evaluated using these test methods, and the range of potential mechanisms of acute oral toxicity? If not, then why? Are additional substances needed to adequately evaluate reliability and accuracy? Are there any other classes of chemicals with known mechanisms of toxicity that may not be detected by the cell culture system that should be tested in future studies. If so, then what specific substances or types of substances are needed? If not, are there redundancies in the types of chemicals evaluated that might be eliminated?

4) Section 6 of the BRD evaluates the accuracy (e.g., concordance) of the NRU basal cytotoxicity test methods for estimating the rodent acute oral LD_{50}, which is used to determine, as part of a weight-of-evidence approach, the starting dose for acute oral toxicity test methods (i.e., the UDP and ATC). Are the strengths and limitations of the NRU basal cytotoxicity test methods, including those applicable to specific chemical classes, chemicals with known mechanisms of toxicity, or to substances with certain physicochemical properties, adequately identified? If not, what additional data and/or analyses are needed?

5) In Section 7 of the BRD, are the analyses regarding the intra- and inter-laboratory reproducibility of the NRU test methods appropriate? If not, then what other analyses should be conducted? Has this section of the BRD adequately elucidated any associations between intra- or inter-laboratory reproducibility and chemical classes, chemical properties, or potency categories?

6) In Section 10 of the BRD:

- Is the extent to which the NRU test methods will reduce animal use in *in vivo* acute toxicity testing adequately characterized and discussed? If not, then what should be added?
- Is the extent to which the NRU test methods will refine (reduce or eliminate pain or distress, including deaths) animal use in *in vivo* acute toxicity testing adequately characterized and discussed? If not, then elaborate on what should be added?
- Is the range of chemical properties and potencies of substances for which this approach may be useful adequately described and are the limitations of the approach adequately delineated?

7) In Section 11 of the BRD, are cost and time to conduct a NRU test method study, and the potential savings in the cost and time to conduct the *in vivo* acute oral toxicity test when preceded by the NRU test method, adequately characterized?

B. Draft ICCVAM Test Method Recommendations

1) Does the Panel agree that the available data support the ICCVAM draft recommendations for these test methods in terms of the:
   - proposed test method usefulness and limitations
   - proposed recommended standardized protocols
   - proposed test method performance standards
   - proposed additional studies

   If not, then why? What recommendations would the Panel make?